The Prokaryote-Eukaryote Dichotomy: Meanings and Mythology

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INTRODUCTION

The prokaryote-eukaryote distinction is perhaps the most well-known fundamental dichotomy in biology, taught in textbooks from high school to university. In regard to organization, the cleavage between what we call prokaryotes and eukaryotes is profound; far greater than that between protist, plant, and animal. Only the eukaryotic form has given rise to all the animal and plant life we see around us. The saltational difference cannot be overstated, for without the emergence of the eukaryotic form we would not be here to discuss it.

The prokaryote-eukaryote dichotomy was indeed universally accepted as a natural order of things until bacterial taxonomy based on evolutionary relationships was revitalized and reformed in the 1970s with the emergence of rRNA phylogenetics. Based on those data and congruent biochemical comparisons, a fundamental trilogy, three domains or superkingdoms of Archaea, Eubacteria, and Eucarya were proposed to replace the previous bifurcation of life forms. At the molecular and biochemical levels, the difference between Archaea and Eubacteria is held to be far greater than that between a human and a plant.

Drawing on documents both published and archival, this paper aims to understand how the prokaryote-eukaryote dichotomy was constructed, the purposes it served, and what it implied in terms of classification and phylogeny. In doing so I first show how the concept was attributed to Edouard Chatton and the context in which he introduced the terms. Following, I examine the milieu in which the terms were reintroduced into biology in 1962. Finally, I study the discourse over the subsequent decade to understand how the organizational dichotomy took on the form of a natural classification and how it was confronted with the rise of rRNA-based phylogenetics.

THE TALE OF EDOUARD CHATTON

In their famed paper of 1962, "The Concept of a Bacterium," Roger Stanier (1916–1982) and C. B. van Niel (1897–

1985) emphasized that the nature and relationships of bacteria, debated since the earliest days of bacteriology, remained unresolved. The authors lamented (53), "Any good biologist finds it intellectually distressing to devote his life to the study of a group that cannot be readily and satisfactorily defined in biological terms; and the abiding intellectual scandal of bacteriology has been the absence of a clear concept of a bacterium." Certainly, they acknowledged, a few of their predecessors had also known that the cell structure of bacteria and blue-green algae was different from that of other organisms, and thus they introduced Edouard Chatton (1883–1947). But they explained that a satisfactory description of the difference could be articulated only after the revolutionary advances in knowledge of cellular organization which followed the development of new techniques after of the Second World War. "It is now clear," they proclaimed (53), "that among organisms there are two different organizational patterns of cells, which Chatton (1937) called, with singular prescience, the eukaryotic and procaryotic type. The distinctive property of bacteria and blue-green algae is the prokaryotic nature of their cells. It is on this basis that they can be clearly segregated from all other protists (namely, other algae, protozoa, and fungi), which have eucaryotic cells."

Thus, lost and neglected, the "singular" insight of Edouard Chatton, like that of Gregor Mendel, was rediscovered decades later. Since that time, the so-called "singular prescience" of Chatton has been echoed in many papers, and it has become accepted that Chatton clearly distinguished between two superkingdoms:

> Chatton (1937) had proposed a most appropriate conceptual basis for taxa at the highest level by recognizing two general patterns of cellular organization- the procaryotes and the eukaryotes. The truth of this prescient generalization was recognized by Stanier (1961) and is now amply supported by a wealth of data derived from comparative cytology involving microscopical, biochemical and physiological approaches (37).

Although foreshadowed by suggestions made by earlier authors, by far the most important advance made in our understanding of the living world as a whole was the realization by Chatton (1937) that there

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are two major groups of organisms, the prokaryotes (bacteria) and the eukaryotes (organisms with nucleated cells). This classification was confirmed and made more widely known by Stanier and van Niel, and it was universally accepted by biologists until recently (33).

The Prokaryote/Eukaryote nomenclature had been proposed by Chatton in 1937 to classify living organisms into two major groups: prokaryotes (bacteria) and eukaryotes (organisms with nucleated cells). Adopted by Stanier and van Neil this classification was universally accepted by biologists until recently (21).

Since its rediscovery in 1962, Chatton's compendium, *Titres et Travaux Scientifiques*, has come to be a landmark publication in biology. The Science Citation Index indicates that it has now been cited in scientific articles some 69 times. But there are difficulties. First, many (45 of 69) of those citations incorrectly date it as 1937; it was published in 1938. This error might appear insignificant and indeed trivial except that (second) Chatton said very little about the distinction in that work. Indeed, there is only one reference to it, in the following passage: "Protozoologists agree today in considering the flagellated autotrophs the most primitive of the Protozoa possessing a true nucleus, Eucaryotes (a group which also includes the plants and the Metazoans), because they alone have the power to completely synthesize their protoplasm from a mineral milieu. Heterotrophic organisms are therefore dependent on them for their existence as well as on chemotrophic Procarytotes and autotrophs (nitrifying and sulphurous bacteria, Cyanophyceae)" (my translation) (8).

There was no articulation, no "prescient generalization," and certainly no "singular prescience" in 1938. In France, *Titres et Travaux Scientifiques* was compiled when candidates were attempting to be elected to an academic body; sometimes a university, very often to the *Académie des Sciences*. Historically, these documents are very interesting and informative, not only about the biography and production of a scientist, but also on self-presentation strategies and conversely on what was valued by institutions and *les grands électeurs*. Chatton's *Titres et Travaux Scientifiques* was an overview of teaching, awards, and predominantly his research on ciliates. In 1937, he was appointed to a chair in marine biology in the *Faculté des Sciences*, Paris, director of the Arago Laboratory at Banyul-sur-Mer on the southern Mediterranean coast of France on the Spanish border, and director of the Biological Station of Ville-Franche-sur-Mer.

That Chatton made no announcement about his prokaryote-eukaryote distinction is significant. The quotation above occurs in the midst of a discussion of a main theme of his cytological research on the question of "the genetic continuity of the ciliary apparatus," the evolution of the kinetosomes, and centrioles. Chatton insisted that kinetosomes were primitive and autonomous organelles, not of nuclear origin, and that centrioles were modified kinetosomes, not the inverse, as was commonly believed. They were at first organs of motility that were later used in mitosis. Research on the reproduction of centrioles and kinetosomes and on their role in morphogenesis has a complex history (42). Chatton's studies were done in the 1920s, especially with his student André Lwoff (1902–1994), who in 1950 published his well-known book *Problems of Mor-*

phogenesis in Ciliates: The Kinetosomes in Development, Reproduction and Evolution (30). Lwoff had used Chatton's terms prokaryote and eukaryote in some of his own publications, and he recommended them to Stanier in 1961.

Chatton first used the words in a much less commonly cited paper of 1925 "*Pansporella perplex*: Reflections on the Biology and Phylogeny of the Protozoa" (only 14 references to this paper in scientific articles over the past four decades) (7). Perhaps in this article one might expect to find an announcement of a new classification of the living world and clear definitions of his terms. Yet here too this is far from the case. In fact, he wrote virtually nothing about them.

At the center of this work is the life history of the amoeba, *Pansporella*, a parasite in the intestines of *Daphnia*, which Chatton discovered as a student in 1906 and later researched at the Institute Pasteur before the First World War. (Though it was published in 1925, he wrote the paper in 1923 at the University of Strasbourg.) As the subtitle suggests, Chatton made some general comments on phylogeny, about primitive flagellated protozoa, "protomastigiées" (protomastigotes), and it is in this context that he used the terms eukaryotes and procaryotes. The words appear only in two figures at the end of his paper when he is discussing "the place of Pansporella among the Amoebiens: the Sporamoebidae group." Traditional classification placed the sporamoebians between the synamoebiens and the entamoebiens, but Chatton insisted that the latter two groups were derived from the first (7).

In his first figure, entitled "*Essai de Classification des Protistes*," he grouped Cyanophycaea, Bacteriacae, and Spirocheatacae as procaryotes. At the base of eucaryotes are three taxa: Mastigiae, Ciliae, and Cnidiae. In his second figure, entitled "*Essai sur la Phylogénie des Protistes*," he placed Cyanophycaeae (blue-green algae) at the base of the tree leading to eukaryotes, the earliest of which were primitive flagellated forms, protomastigotes. Spirochetes and Bacteriaceae are drawn as side branches from Cyanophytes (7).

Chatton did not ascribe prokaryotes to a superkingdom. Instead, he used the word Protozoa, which Richard Owen had proposed for the kingdom, and Ernst Heackel's word Protists interchangeably for the bacteria and other unicellular organisms; sometimes he referred to bacteria by the old name, "schizophytes" (fission plants). Again, the classification of bacteria was not problematic for him; it was not on his agenda. His concern was elsewhere: on the evolution of primitive flagellated protozoa, "protomastigiées." "One can hardly conceive of the passage from Schizophyte to the Protozoa other than by a flagellated form. The hypothesis of Bacteriés—primitive beings has a necessary corollary, in our present state of knowledge, that of primitive flagellated-Protozoa" (7).

The difference between prokaryote and eukaryote was simply not a major insight as far as Chatton was concerned. Many microscopists since the days of Ernst Haeckel had recognized a difference between bacteria (and blue-green algae) which lacked a true nucleus and other protists (which contained a nucleus) (44). In his *Generelle Morphologie der Organismen* (1866), Haeckel designated the third living kingdom, the Protista, the first living creatures. They included the "Protozoa" and "Protophyta" as well as "Protista Neutralia," those ancestral to neither plant nor animal. Haeckel placed the bacteria in the order Moneres (later Monera) at "the lowest stage of the

protist kingdom." Bacteria were unique, he argued, because unlike other protists, they possessed no nucleus. They were as different from nucleated cells as "a hydra was from a vertebrate" or "a simple alga from a palm" (23).

Later, in his *Wonders of Life* (1904), Haeckel included the Cyanophyceae (blue-green algae) among the Moneras with the bacteria. Though they were usually classified as a class of algae, he asserted that they lacked a nucleus and that the only real comparison between them and plants was with the chromatophores (chromatella, chloroplasts). Thus, he suggested that the plant cell evolved as "a symbiosis between a plasmodomonous green and plasmophagus not-green companions" (24). Such ideas about symbiosis were not uncommon since the 1880s, though they were only systematically investigated a century later when suitable techniques were developed.

Whether bacteria and blue-green algae possessed a true nucleus and whether they divided in the manner of other cellular organisms remained controversial well into the 20th century (16). Some maintained that while bacteria had no nuclei and exhibited no true mitotic division, blue-green algae did contain a nucleus-like body which may undergo a simple form of mitotic division (28). Protists remained divided among botanists and zoologists as lower plants and lower animals. Bacteria and the green flagellates from which plants were thought to have descended were the subjects of botanists, and the colorless flagellata, the protozoa, heliozoa, foraminifera, and infusoria were the subjects of zoologists.

Whether or not one could have a natural classification of bacteria, one based on evolutionary relationships, was intensely debated in the early 20th century (44). American botanist Edwin Copeland argued in 1927 that a plant kingdom which included the bacteria was "no more natural than a kingdom of the stones" (9). In 1938, his son, Herbert Faulkner Copeland, wrote a more detailed paper (10), and in 1956, a book (11), proposing that Haeckel's Monera be granted their own kingdom on the grounds that they were "the comparatively little modified descendants of whatever single form of life appeared on earth, and that they were sharply distinguished from protists by the absence of nuclei" (10). Hence, there were four natural kingdoms: Monera, Protista, Plantae, and Animalia.

In 1941, Stanier and van Niel, who at that time insisted that there be a natural classification for bacteria, followed Copeland in assigning the bacteria and the blue-green algae to the kingdom Monera based on three common features: absence of true nuclei, absence of sexual reproduction, and absence of plastids (52). But when, in 1962, Stanier and van Niel reintroduced the term procaryote and articulated a concept of the bacterium, they made no reference to the kingdom Monera (53). Significantly, by that time, as I shall explain, they had lost hope for bacterial phylogenetics.

REINTRODUCING THE WORDS

The molecular biology of the gene and the deployment of the electron microscope after the Second World War permitted refinement in the concept of a bacterium in terms of genetics, biochemistry, and morphology. That improvement began with distinguishing the virus from small bacteria and from any cellular organism. Since the nineteenth century, bacteria had been removed using filters, but some infectious agents

were so small as to pass through a bacterial filter. They were called filterable viruses. Other small, obligate, parasitic bacteria of the rickettsial type, barely resolvable by the light microscope, were often thought to be transitional between filterable virus and the typical bacterium. Thus, bacteria were thought to range in size from those the size of some algae to those the size of filterable viruses. As late as 1948, the editors of *Bergey's Manual of Determinative Bacteriology* suggested a new kingdom, *Protophyta*, which would include both bacteria and viruses (4).

In 1957, André Lwoff articulated major differences between viruses and bacteria, based on molecular structure and physiology (29). The virus contained either RNA or DNA enclosed in a coat of protein, and it possessed few if any enzymes except those concerned with attachment to and penetration into the host cell. The virus was not a cell and did not reproduce by division like a cell. Its replication occurred only within a susceptible cell, which always contains both DNA and RNA and an array of different proteins endowed with enzymatic functions mainly concerned with the generation of ATP and the synthesis of varied organic constituents of the cell from chemical compounds in the environment. "Viruses should be treated as viruses," Lwoff (29) concluded, "because viruses are viruses." There were no biological entities which could properly be described as transitional between a virus and a cellular organism, and the differences between them were of such a nature that it was indeed difficult to visualize any kind of intermediate organization.

Stanier and van Niel's paper "The Concept of a Bacterium" followed from that of Lwoff on the virus. No question that electron microscopy and genetics helped to clarify some issues about the structure and function of bacteria. In 1946, Joshua Lederberg and Edward Tatum reported that bacteria had genes and exhibited sexual recombination (27). They could no longer be defined as asexual organisms. Nonetheless, Stanier and van Niel still could do little more than define the procaryotes in negative terms in relation to eukaryotes. Eukaryotes had a membrane-bound nucleus, a cytoskeleton, an intricate system of internal membranes, mitochondria that perform respiration, and, in the case of plants, chloroplasts. Bacteria (prokaryotes) were smaller and lacked all of these structures; they lacked mitosis: "The principal distinguishing features of the procaryotic cell are: 1 absence of internal membranes which separate the resting nucleus from the cytoplasm, and isolate the enzymatic machinery of photosynthesis and of respiration in specific organelles; 2 nuclear division by fission, not by mitosis, a character possibly related to the presence of a single structure which carries all the genetic information of the cell; and 3 the presence of a cell wall which contains a specific mucopeptide as its strengthening element" (53).

Just as there would be no transitional forms between viruses and bacteria, there would be no transitional forms between bacteria and all other organisms. Stanier, Michael Douderoff, and Edward Adelberg declared, in the second edition of *The Microbial World* (1963), that "In fact, this basic divergence in cellular structure, which separates the bacteria and blue-green algae from all other cellular organisms, represents the greatest single evolutionary discontinuity to be found in the present-day world" (55).

PHYLOGENY ABANDONED

There was still another aspect to the context in which the revitalized dichotomy was launched: the perennial debate over natural versus artificial classification of bacteria (44). Could one have a natural, phylogenetic classification? Or should the classification of bacteria be determinative and based solely on usefulness, like the organization of library books? If the former, what traits could be used: physiological or morphological or both? The classifications of plants and animals were based on comparative anatomy and embryology. Bacteria lacked complex morphological traits, and though they showed enormous physiological diversity, it was difficult to discern which were old and which were recent adaptations. By the early 1920s, many bacteriologists had give up on phylogeny, including the editorial board of the first edition of *Bergey's Manual*. They opted for a useful, reasonably stable, unnatural classification instead of a phylogenetic classification, which they perceived to be forever speculative and continually changing.

van Niel and Stanier's own attitudes towards classifying bacteria took sharp turns. Early in the twentieth century, the Delft school held out for a taxonomy reflecting evolutionary relationships. In 1936 van Niel and his professor Albert Jan Kluyver reasoned that a phylogenetic classification be based, in the first instance, on increased morphological complexity (26). Stanier and van Niel (1941) had reiterated the arguments for a phylogenetic classification in a trenchant assessment of *Bergey's Manual*, which they ridiculed for rejecting a phylogenetic approach and for offering a completely inadequate definition of bacteria (52).

Despite public optimism, van Niel was still far from confident about a natural bacterial classification. He privately conveyed his despair about the whole field of microbiology in a letter to Stanier dated 13 August 1941, early in their relationship: "Many, many years ago I often went around with a sense of futility of all our (my) efforts. It made me sick to go around in the laboratory (this was in Delft) and talk and think about names and relations of microorganisms; about the fate of substrates and hydrogen atoms, about—well about everything. During those periods I would go home after a day at the lab, and wish that I might be employed somewhere as a high-school teacher. Not primarily because I liked that better. But simply because it would give me some assurances that what I was doing was considered worth-while" (C. B. van Niel to Stanier: 13 August 1941; Stanier papers, National Archives of Canada, Ottawa).

When van Niel addressed bacterial classification at the famous Cold Spring Harbor Symposium of 1946, his support for the schemes he, Kluyver, and Stanier had proposed years earlier had weakened (58). He conceded that bacteriologists' "fragmentary knowledge of bacterial phylogeny is far from sufficient to construct anything like a complete system. Even for a general outline along phylogenetic lines, the available information is entirely inadequate. Much of this is, of course, the result of the paucity of characteristics, especially those of a developmental nature" (58). Nonetheless, he avowed, "the search for a basis upon which a 'natural system' can be constructed must continue" (58).

In 1955 van Niel, following Sergei Winogradsky (1856 to 1953), disavowed bacterial phylogenetics (60). Winogradsky had asserted that phylogenetic classification was simply "im-

possible to apply to bacteria" (63). Both he and van Niel reminded bacteriologists that the order of things in *Bergey's Manual* in terms of species, genera, tribes, families, and orders was only a facade. To avoid the delusion that it represented a natural ordering, both he and van Niel suggested using the term biotypes instead of species and using common names such as sulfur bacteria, photosynthetic bacteria, and nitrogen-fixing bacteria" instead of Latin names with their phylogenetic implications (60).

That a natural phylogeny of bacteria was impossible was reiterated by Stanier, Doudoroff, and Adelberg in the first edition of *The Microbial World* (1957) (54): ". . .the construction of the broad outlines of a natural system of bacterial classification involves much guesswork and affords the possibility for endless unprofitable disputes between the holders of different views about bacterial evolution. An eminent contemporary bacteriologist, van Niel, who is noted for his taxonomic studies on several groups of bacteria, has expressed the opinion that it is a waste of time to attempt a natural system of classification for bacteria, and that bacteriologists should concentrate instead on the more humble practical task of devising determinative keys to provide the easiest possible identification of species and genera. This opinion, based on a clear recognition and acceptance of our ignorance concerning bacterial evolution, probably represents the soundest approach to bacterial classification, but it has not gained universal acceptance."

Stanier turned his youthful polemics of the early 1940s around 180° when, in the second edition of *The Microbial World* (1963), he, Doudoroff, and Adelberg criticized the editors of *Bergey's Manual* for actually attempting a natural classification, one based on both physiological and morphological characters. But, "if one overlooks its unverifiable phylogenetic implications, *Bergey's Manual* does serve a very useful purpose as a work of reference" (55). Stanier, Doudoroff, and Adelberg confidently asserted that bacteria were monophyletic on account of the prokaryotic structure but that the macroevolutionary relationships of bacteria simply could not be arranged phylogenetically (55): "All these organisms share the distinctive structural properties associated with the procaryotic cell . . . , and we can therefore safely infer a common origin for the whole group in the remote evolutionary past; we can also discern four principal subgroups, blue-green algae, myxobacteria, spirochetes, and eubacteria, which seem to be distinct from one another. . . Beyond this point, however, any systematic attempt to construct a detailed scheme of natural relationships becomes the purest speculation, completely unsupported by any sort of evidence." Thus, they concluded that "the ultimate scientific goal of biological classification cannot be achieved in the case of bacteria" (55).

That bacteria represented a monophyletic group had been an implicit assumption for Stanier and van Niel when, in 1941, they supported the new kingdom Monera. In 1949 van Niel also stated some reasons for the monophyletic origin of life based on Kluyver's concept of "biochemical unity" (59):

> These two aspects of life—its constancy and variability—are reflected in many ways. From the point of view of comparative biochemistry, the constancy finds its expression and counterpart in the unity of the fundamental biochemical mechanisms, that is

Kluyver's concept of the "unity of biochemistry." This, today, is also the most compelling argument in favor of a monophyletic origin of life. The variability by comparison, can be related to the existing biochemical diversity, so glaringly apparent especially among microorganisms, and it represents the numerous directions in which adaptations to a new environment have become established. The persistence of so many patterns, like variations of a theme, drives home the importance of individuality, without which there could be no differences—nor evolution.

If bacteria were polyphyletic, then the category prokaryote would have no evolutionary or phylogenetic meaning. It would merely be an illusion, as in the nineteenth-century assumption that rhinos, hippos, and elephants descended from a single large ancestor. (It is now known that each of these animals evolved from a separate small ancestor, and the common ancestor of all of them was small and slightly built, with presumably thin skin and fur.) Those animals share derived characters which originated several times by convergence. When defined negatively in terms of what they lacked, the taxon prokaryote might well be similar to the grouping invertebrate, which includes such diverse creatures as insects and worms.

Bacteria had always been defined largely in negative terms: they lacked a nucleus, lacked mitosis, lacked sex. For Stanier, however, the prokaryote-eukaryote distinction seemed to somehow resolve the problem, as he commented in 1982 (51): "Indeed that was the catch about it. As recently as 40 years ago, Stanier and van Niel (1941) could do little better, in an attempt to define collectively these two groups. The issue was at last resolved (at least, to the author's satisfaction) by the discovery of a major evolutionary discontinuity, at the cellular level, among all biological systems. I allude to the distinction of two super-kingdoms, eukaryotes and prokaryotes. I think it is profoundly significant that the fundamental difference between eukaryotes and prokaryotes could not be rigorously formulated prior to approximately 1960. It was André Lwoff who proposed these two names during a discussion I had with him in 1961. He revived these historically appropriate names from oblivion, citing as his authority an equally obscure and rare publication from the great French protozoologist Edouard Chatton (1938). Nine years later, I failed to mention Chatton's proposal (Stanier 1970) in the course of preparing a chapter...a sure sign that these terms have entered history... Quite independently (and about 20 years later than Chatton) Dougherty (1957) had proposed the prokaryotic-eukaryotic dichotomy."

Three issues are raised above. First, despite his remarks about his omission in the chapter he wrote in 1970, actually the year following their famed paper of 1962, in the second edition of *The Microbial World*, Stanier, Douderoff, and Adelberg reintroduce the terms with no reference to Chatton but as effectively a neologism of their own coinage: "[T]here are two quite different kinds of cells among existing organisms. The more highly evolved type, which we shall term the *eucaryotic cell*, is the unit of structure of all plants and animals and in several large groups of protists: fungi, protozoa, and most algae. A much simpler kind of cell, which we shall term the *procaryotic cell*, is the unit of structure in *all bacteria* and in one group of algae, the *blue-green algae*" (55).

Stanier's reference to Doughtery requires explanation. In 1955 Ellsworth Doughtery endorsed the kingdom Monera (17). Two years later he used the words eukaryon (Greek: true kernel) for the nucleus of "higher organisms" and prokaryon (Greek: before kernel) for the moneran nucleus (18). Prokaryotic and eukaryotic in his usage meant "the condition of possessing prokarya or eukarya." They were not meant to be taxonomic terms but only a way of assigning words to the primitive nucleus of bacteria and "the more organized nucleus" of plants, animals, and protists. That is, they were organellar terms. Doughtery He also called for new words to differentiate between "the 'flagellum' and homologous organelles of higher organisms and the 'flagellum' (vibratory organelle) of bacteria": pecilokont (Greek intricate pole) and proterokont (Greek: earlier pole), respectively (18).

Third, the question about the negative characteristics of the prokaryotes was not resolved in the 1960s. There was no such talk about a natural phylogeny or a superkingdom when Stanier and van Neil introduced the words procaryote and eucaryote to English literature. To understand the evolution of their views on the matter, I turn to archival data and correspondence.

BEHIND THE SCENES

Stanier drafted an outline of "The Concept of a Bacterium" in 1961 when he was a visiting scientist at the Pasteur Institute in Paris. That year he had used the terms in a paper written in French for the *Annales de l'Institut Pasteur*. In it, he referred to Lwoff's arguments about the difference between a virus and a cell and that bacteria and blue-green algae share a prokaryotic structure. Arguing that the most general organizational differences between prokaryotic cells and eukaryotic cells is that the former lack a nuclear membrane, he concluded that "One can define the bacteria and establish their place in the living world by the structure of the procaryotic cell" (47).

He sent an outline of his newly proposed paper to his mentor, van Niel, with a request to collaborate in the festschrift for famed Czech microbiologist Ernst Georg Pringshiem (1881–1970). Stanier wanted the paper to be neutral with regard to taxonomic implications. Van Neil responded favorably to the request to collaborate, but he doubted that the classifications of large groups in their paper had anything more than utilitarian implications:

> The proposition you made is certainly a most attractive one, and I should much prefer to have a joint paper with you for the Pringsheim volume than something else. Hence, in principle, I should like to see what can be done, and how best to do it. After considering the outline for the three sections that you had sketched, I am somewhat doubtful about the claim in the first paragraph of your letter that the paper would be "without taxonomic implications." Is not the projected Section 3 rather clearly involved with this problem? Granted that separations of large groups can be made on the basis of the mechanisms of locomotion, and perhaps, of the Gram stain and its underlying chemical differences, would the resulting groups really have more than utilitarian significance? I am not yet convinced that this would be so. And of

course, as you say, the problem of the permanently immotile types remains an extremely difficult one; it has always been so. However, it is possible that matters of this sort will become clearer during the writing. Anyhow, I would personally consider it a great pleasure once again to do a joint paper with you (C. B. van Niel to Stanier, February 1961, National Archives of Canada, MG 31, accession J35, vol. 6).

Stanier gave van Niel a copy of his manuscript at the Chicago meetings in the spring of 1961. Van Niel later wrote to say that on the whole he found the paper to be excellent but was concerned with sharpening two key main points: the distinction between viruses and bacteria, and the homology of the structures of bacteria and blue-green algae:

> The first one is in connection with the distinction between bacteria and viruses. While I agree that, on the basis of Andre's [Lwoff] definition, such a separation presents no difficulties, it seems to me that logically the approach could be improved. As is so often the case, a definition is very helpful, but only if it be accepted by others. This implies that it might be better to lead up to the definition, rather than start with it, as is done in the paper. What one should like to do is to emphasize that among the biota of exceedingly small size (perhaps to be indicated as "filterable" in keeping with the original concept of a virus) two distinct groups can be recognized: those with the multiplicity of structures and functions encountered in the PPLO group, the Rickettsias, etc., and those comprising entities composed of a single type of nucleic acid. If it be further emphasized that the latter are duplicated by the host cells, rather than multiply as autonomous units, the distinction can be made even more clear-cut. Once this has been done—it should not be hard for the reader to recognize the fundamental distinctions—the use of names and definitions can properly be advocated.
>
> On pg 8 you mention that the homology of structures in bacteria and bg. Algae is supported by the impressive result of studies in the area of bacterial genetics. Later, however, it is stated that the latter applies virtually exclusively to E. coli; and you also state that thus far nothing is known concerning the genetics of bg. Algae. Thus, it would seem wise not to make too much of that point at this particular place.
>
> Do you believe wholeheartedly that bacterial and bluegreen algae chromatophores will never be shown to be structures with a membrane? Granting that such membranes have not been shown to occur, I nevertheless have certain reservations to make this into a sort of pontifical dogma. Would you agree to phrase this a little less absolutistically?
>
> . . . I find it difficult to see what else you wanted to include. Because at the moment I just can-not do more than jot down some notions that have occurred to me while rereading the manuscript, I hope you will let me know what I may expect next. (C. B. van Niel

to Stanier, 19 May 1961, National Archives of Canada, MG 31, accession J35, vol. 6).

Their paper was completed by October, and van Niel was delighted. As he wrote in a letter to Stanier and his wife, Germaine Cohn-Bazire: "It was wonderful to see you again, and I am very, very grateful that you have been willing to let me be a coauthor of the paper on 'The Concept of a Bacterium.' During the week I have thought about it, off and on, and believe that it is really quite good. It does not seem likely that a final reading will cause me to change my mind about it, and I don't expect that I'll want to propose any significant changes." (C. B. van Niel to R.Y Stanier and Germaine Cohn-Stanier, 2 October 1961, National Archives of Canada, MG 31, accession J35, vol. 6).

In the concluding paragraph of their famed paper of 1962, they referred to the arguments of Pringsheim in regard to blue-green algae (53): "As Pringsheim (1949) has so persuasively argued, the bacteria and blue-green algae encompass a number of distinct major groups, which do not now appear to be closely related to one another; their only common character is that they are procaryotic. It thus appears that the procaryotic cell has provided a structural framework for the evolutionary development of a wide variety of microorganisms. . . . If we look at the microbial world in its entirety, we can now see that evolutionary diversification. . .has taken place on two distinct levels of cellular organization."

Yet Pringsheim's views differed from those of Stanier and van Niel in two fundamental respects. First, he believed that the taxon Monera was polyphyletic, and second, he continued to urge a natural classification. The main thrust of Pringsheim's extensive review of 1949 is the question of whether the Myxophyceae (cyanobacteria) are related to the Bacteria (39). But Pringsheim was skeptical that the bacteria and the blue-green algae (Myxophyceae) had a common ancestor. He noted that the kingdom Monera, which Stanier and van Niel had supported in 1941, was defined negatively. It was entirely possible, perhaps likely, he argued, that the similarities between blue-green algae and bacteria resulted from convergent evolution. "Stanier and van Niel (1941). . .believe that Bacteria and blue-green algae have originated from common ancestors and summarize their common characteristics as follows: (1) absence of true nuclei, (2) absence of sexual reproduction, (3) absence of plastids. . . The entirely negative characteristics upon which this group [Monera] is based should be noted, and the possibility of convergent evolution of the two classes be seriously considered" (39).

The issue here is not whether Pringsheim was correct about a lack of affinity between cyanobacteria and bacteria but his attitude about the need to demonstrate such relations. Far from taking the monophyly of Monera at face value, Pringsheim noted how molecular biology might offer the appropriate evidence for distinguishing the larger taxonomic groups: "Modern methods of extracting specific proteins and other compounds of high molecular weight may eventually afford the clue to the problems above indicated" (39). Nonetheless, when the prokaryote was defined in 1962, many microbiologists eagerly accepted it, not just as an organizational distinction, but as a phylogenetic one.

MONERA—A KINGDOM LACKING

As formulated by Stanier and van Niel in 1962, the prokaryote-eukaryote distinction was an organizational distinction, conveying the hierarchical nature of biological organization. However, the meaning of the prokaryote-eukaryote dichotomy quickly changed so as to signify a phylogenetic distinction. The publication of the prokaryote concept was met with enthusiastic approbation, and leading microbiologists believed that the prokaryotes should be immediately recognized with their own kingdom.

Indeed, the rhetorical discovery (or "rediscovery") of the prokaryotes served to confirm and legitimate the kingdom Monera proposed but not fully recognized decades earlier. Thus, some of the system builders, such as ecologist Robert Whittaker, changed their views about kingdoms based on Stanier and van Niel's paper. In 1959, Whittaker had not included Copeland's Monera as a kingdom, and recognizing an ecological division between autotrophs and heterotrophs, he added the kingdom Fungi to that of Protista (with subkingdoms Monera and Eunculeata), Plantae, and Animalia (61). But a decade later, following the presentation of the prokaryote-eukaryote dichotomy, based on structural organization, he recognized the kingdom Monera. As he commented, "These contrasts between the prokaryotic cells of bacteria and blue-green algae, and the eukaryotic cells of other organisms, define the clearest, most effective discontinuous separation of levels of organization in the living world . . .the difference between procaryotes and eukaryotes remains a line of division deserving recognition in a current system of broad classification" (63). Thus, adding Monera to fungi (mycota), protists, plants, and animals, he advocated five kingdoms. What Whittaker found to be so persuasive in regard to recognizing a new kingdom of Monera were in fact all negative differences between bacterial cells and those of other organisms (62):

> Cells of bacteria and blue-green algae lack of mitochondria and plastids, nuclear membranes and mitotic spindles, the endoplasmic reticulum, and Golgi apparatus, vacuoles, and advanced (9 + 2 strand) flagella, among the organelles characteristic of the cells of other organisms. Nuclear material is probably a single strand of DNA without histones, dividing by means other than mitosis; sexual reproduction is apparently both infrequent and incomplete in the sense that only partial recombination of genetic material of cells may result from bacterial conjugation and other processes. Bacteria and blue-green algae also resemble one another and differ from other organisms in biochemical characteristics, including their method of ornithine synthesis, the apparently limited occurrence of sterols, sensitivity to antibiotics, and cell wall composition.

Whittaker made no apologies about defining the group negatively, nor did he query whether Monera was a monophyletic kingdom. He did, however, question whether the other four kingdoms were monophyletic. In fact, he confidently asserted, "The three higher kingdoms [Plantae, Fungi, and Animalia] are polyphyletic." And he suspected the same may be true for Protista (62). Whittaker had few qualms about it: "Monophyly

is a principal value of systematics, but like other values is not absolute and will not always be followed to the sacrifice of other objectives" (62).

Some members of the editorial board of *Bergey's Manual* were equally enthusiastic about the prokaryote-eukaryote dichotomy. R. G. E. Murray wanted the major structural or organizational differences between prokaryotes and eukaryotes to be recognized immediately by a taxonomic, phylogenetic separation. The same year that Stanier and van Niel introduced the terms, Murray had argued on the same morphological grounds that the Monera be promoted to the rank of a kingdom (Mychota) of bacteria and blue-green algae (35). He sent a preprint of his paper to Stanier and wrote to him in May 1962 about "The Concept of the Bacterium" that he admired "the terse and well-disciplined definition of principles" that he had attained. Murray explained that he himself had expressed similar views but that he did not understand why Stanier was no longer willing to defend his attitude of 1941 "that a major difference in organization deserves to be recognized by a taxonomic separation." (R. G. E. Murray to R. Y. Stanier, 15 May 1962, National Archives of Canada, MG 31, accession J35, vol. 6).

Stanier replied that although he had no objection to a new kingdom, he and van Niel considered phylogenetic classification a meaningless exercise: "I should certainly not object to setting up a separate kingdom for the prokaryotic microorganisms if such an operation would serve as a handy device for emphasizing the fundamental differences between these types and organisms that possess a eukaryotic cellular organization. All the introductory statement meant to imply is that both van Niel and I now consider detailed system building at the microbial level to be an essentially meaningless operation, since there is so very little information that can be drawn on for the purposes of phylogenetic reconstruction. For this reason I prefer to use common names rather than Latin ones for every bacterial group above the level of genus" (R. Y. Stanier to R. G. E. Murray, 21 May 1962, National Archives of Canada, MG 31, accession J35, vol. 6).

The monophyly of the prokaryotes was not an issue for Murray; the only real question was whether Monera or Procaryota should be used for the new kingdom. In 1968, he proposed Procaryotae as a taxon "at the highest level" and described it as "a kingdom of microbes. . .characterized by the possession of nucleoplasm devoid of basic protein and not bounded from cytoplasm by a nuclear membrane." He suggested Eucaryotae as a possible taxon at the same level to include other protists, plants, and animals (36). The following year, A. Allsopp at the University of Manchester suggested that Procaryota and Eucaryota might be given the status of "superkingdom" (1).

Still, other members of the editorial board of *Bergey's Manual* were more cautious. R. E. Buchanan, chairman of the Bergey's Manual board of editors, had four concerns. First, he noted the almost entirely negative characteristics by which the group was identified; second, he was not completely confident that blue-green algae should be identified as prokaryotes; third, he was not certain how viruses could be completely distinguished from bacteria and as such would no longer be included with bacteria in the kingdom *Protophyta*, which *Bergey's Manual* had suggested in 1948 (2). These concerns aside, the only remain-

ing problem was what should be the correct name for the kingdom, Prokaryota or Monera. Buchanan wrote to Stanford botanist Peter Raven about these matters in 1970, informing him that the next edition of *Bergey's Manual* was planning to introduce the bacteria with a concise statement of their relationships.

Raven agreed with Buchanan about the negative characterization of the prokaryote but noted that the ribosomes of prokaryotes were distinctive. Ribosomes were composed of two subunits, both of which contain RNA and protein. In prokaryotes the smaller subunit was 30S and the larger was 50S. In eukaryotes the two subunits were larger, 40S and 60S. (The designations 30S and 50S refer to the rates at which each of these bodies sediment in an ultracentrifuge.) Raven did not consider the blue-green algae to be more distinctive as a group than many of the other groups currently regarded as bacteria, and he considered "viruses as a group outside the usual classifications of living organisms" (Peter Raven to R. E. Buchanan, 8 October 1970, National Archives of Canada, MG 31, accession J35, vol. 6). Raven had explained this in his own 1970 book with Helen Curtis (40) and in consultation with his colleague Allen Campbell. He also emphasized that Whittaker's five-kingdom proposal was "widely accepted" and that Monera was the most common name for the prokaryotic kingdom.

Buchanan responded that he had "also studied very carefully Dr. Whittaker's outline of five kingdoms. It is well balanced and thought-provoking" (R. E. Buchanan to Peter Raven, 22 October 1970, National Archives of Canada, MG 31, accession J35, vol. 6). However, he objected to the term Monera for the kingdom, based on his own reading of Haeckel's writings. "Inasmuch as the blue-greens and the bacteria seem to be run together," he would suggest "the use of Procaryote, Procaryotae or Procaryotes as the name of the kingdom." But he was still uncertain how to provide an adequate statement differentiating small obligate parasites such as rickettsiae and chlamydiae on the one hand and viruses on the other. Finally, he inquired as to the fossil evidence about prokaryotic life.

Raven replied that he still preferred the name Monera for the kingdom and explained that there was fossil evidence that prokaryotic organisms have existed far longer than any other kind; they were found in the oldest rocks known, some 3.2 to 3.4 billion years old, whereas the oldest eukaryotic organisms appeared as fossils only about 1.2 billion years ago (Peter Raven to R. E. Buchanan, 3 November 1970, National Archives of Canada, MG 31, accession J35, vol. 6). The Earth was considered to be nearly 5 billion years old, and life probably originated some 4 billion years ago. Therefore, prokaryotes would have existed for approximately 3 billion years, or three quarters of the history of life on earth, before eukaryotes evolved. Raven suggested that viruses, probably as old as life itself, might be regarded as by-products of bacterial reproduction, in which segments of DNA or RNA protected with protein coats spread from cell to cell, directing the host cell's metabolism to reproduce more of the viral DNA or RNA.

Raven wrote to Stanier asking for his advice on the relationship between blue-green algae and bacteria. Were they "merely specialized bacteria that 'hit upon' the system of photosynthesis that has become standard in the green algae and higher plants," as he supposed (Peter Raven to Roger Stanier, 3 November 1970, National Archives of Canada, MG 31, accession J35, vol. 6). Stanier responded:

As a matter of fact, we've been working hard for the past 5 years in the biology of blue-green algae, which has now become my principal field of research. All things considered, I think it is now quite evident that the blue-green algae are not distinguishable from bacteria by any fundamental feature of their cellular organization: their sole distinctive and unique property as procaryotes is the possession of a group-specific photopigment system, and of a photosynthetic machinery which contains type II, as well as type I, reaction centers. Considered as procaryotes, they are just another specialized photosynthetic group, comparable to the green bacteria and to the purple bacteria. Their major evolutionary interest, of course, is connected with the possible origin of the chloroplast. I think the evidence now points inescapably to the conclusion that chloroplasts (and mitochondria) had evolutionary origins distinct from that of the other components of the eukaryotic cell, having arisen from prokaryotic endosymbionts.

The chemical resemblance between blue-green algae and chloroplasts suggest that the chloroplast in a few eukaryotic groups probably had a blue-green algal origin: specifically, in the red algae and the cryptomonoads. I'm not so sure about other types of chloroplasts, and rather like the idea that the green algal higher plant chloropasts and the brown algal chloroplast may have been derived from other groups of O_2-evolving photosynthetic prokaryotic ancestors, now extinct in the free-living state.

In terms of our present insights, accordingly, the assignment of the blue-green algae to the "algae" is just an unfortunate historical accident, though a somewhat ironic one: the botanists, in general reluctant to admit the importance of biochemical characters, were misled by a biochemical and physiological resemblance, which caused them to overlook the fundamental cytological differences between eukaryotic algae and blue-greens.

As to what one might do about this situation in formal taxonomic terms, I don't really care very much, since taxonomic system-building (especially in the realms of the [micro] biological world) isn't an operation that seems very useful (Roger Stanier to Peter Raven, 5 November 1970, National Archives of Canada, MG 31, accession J35, vol. 6).

During the 1970s and 1980s, led by the writings of Lynn Margulis, much attention focused on the question of whether mitochondria and chloroplasts arose as symbionts (32, 41). And although Stanier and van Niel had asserted that "a definition of a bacterium is only possible if one includes the blue-green algae," the acceptance of their bacterial nature was far from straightforward. The word cyanobacteria first appeared in the eighth edition of *Bergey's Manual* in 1974. Stanier's efforts, beginning in the early 1970s, to change the jurisdiction over cyanobacteria from the international Code of Botanical Nomenclature to the International Code of Nomenclature of Bacteria continued to the end of his life in 1982. As Alexander

Glazer commented, "His lack of success offers a spectacular example of the power of traditional form over substance" (21).

During the 1970s, Monera was frequently used for the name for a fifth kingdom, though the editors of the eighth edition of *Bergey's Manual* called the kingdom *Procaryotae*. Murray explained in the introductory chapter (37): "The Bergey's Manual Trust has discussed these names and the various alternatives on many occasions and agreed that *Procaryotae* was the most appropriate, as a plural feminine noun, for such a taxon.

"The assumption of a new Kingdom is both appropriate and helpful to the bacterial taxonomist, but a kingdom including all the eukaryotes would be disturbing to botanists and zoologists causing a realignment of their respective hierarchies. It is probably best to leave matters as they have been expressed above and only recognize, at the moment, the Kingdom *Procaryotae*."

Gunther Stent used the superkingdom of *Prokaryota* and *Eukaryota* in his 1971 text *Molecular Genetics* and summarized the organizational differences between them. Prokaryotes were 1 to 10,000 times smaller, they have 1000th as much DNA as a mammalian cell, the lack a nuclear membrane, the DNA is not combined with protein to form structures like eukaryotic chromosomes, they lack mitosis and meiosis, and they lack mitochondria and centrioles. Thus, he concluded, "there can be little doubt that the simpler prokaryotes are the evolutionary antecedents of the more complex eukaryotes" (56).

Before the 1980s, there were few published criticisms of the prokaryote-eukaryote dichotomy. An exception was the bacteriologist K.A. Bissett at the University of Birmingham, who in 1973 questioned whether bacteria and blue-green algae were really prokaryotic organisms which lack a nuclear membrane and whether they really preceded eukaryotes (3). He argued that the inner layer of the bacterial cell envelope was actually comparable to the nuclear membrane of protists and therefore proposed that "the structure which now represents the cell membrane of bacteria may have originated in the nuclear membrane of an ancestral form." In short, bacteria may be economized forms of eukaryotes that transformed their nuclear membrane. Like many others before him, he pointed to the negative characterization of the prokaryote (3): "It may be that the supposed resemblance between bacteria and blue-green algae in having no nuclear membrane will prove to be as invalid as the previous theory; that they were alike in having no nucleus at all. They are alike mainly in their small size, and in the convergent adaptations that this produces. Positive grouping based on negative criteria, are seldom durable in biology, and separate creation, even of organs, must have some evolutionary background."

Stanier also considered the possibility that eukaryotes preceded prokaryotes, at least in jest. As he commented in 1970 when considering the symbiotic origins of mitochondria and chloroplast (48): "Is the comparative structural simplicity of prokaryotic organisms really indicative of great evolutionary antiquity? In view of their similarities to mitochondria and chloroplasts, it could be argued that they are relatively late products of cellular evolution, which arose through the occasional escape from eukaryotes of organelles which had acquired sufficient autonomy to face life on their own. This is a far-fetched assumption; but I do not think one can afford to dismiss it out of hand."

Stanier remained skeptical of evolutionary arguments, insist-

ing (48) that "Evolutionary speculation constitutes a kind of metascience, which has the same fascination for some biologists that metaphysical speculation possessed for some medieval scholastics. It can be considered a relatively harmless habit, like eating peanuts, unless it assumes the form of an obsession; then it becomes a vice."

Though Stanier may have spoken for many microbiologists, the above assertion was short-lived. Statements about microbial phylogenetics as a failed, disreputable pursuit in 1962 and 1963 occurred at the very time molecular evolution was emerging (14, 15). In 1963 Emmanuel Margoliash and his collaborators had compared similarities and differences in amino acid sequences of cytochrome *c* molecules from horses, humans, pigs, rabbits, chickens, tuna, and baker's yeast to infer phylogenetic relationships (31). Emile Zuckerkandl and Linus Pauling had also pioneered the use of amino acid sequence comparisons to infer evolutionary relationships in primate phylogeny with data from hemoglobin sequences. Their famed paper of 1965 marked the gateway to molecular evolution for many who entered this field (71). The idea had been mentioned by Francis Crick in 1958, a few years before the genetic code was cracked (12): "Biologists should realize that before long we shall have a subject which might be called 'protein taxonomy'—the study of amino acid sequences of proteins of an organism and the comparison of them between species. It can be argued that these sequences are the most delicate expression possible of the phenotype of an organism and that vast amounts of evolutionary information may be hidden away within them."

During the 1960s and 1970s, bacterial taxonomist led by Jozef De Ley also used the GC content of DNA and nucleic acid pairing to classify bacteria (13). Peter Sneath added those new characteristics to myriad others he inputted into his computer-assisted numerical taxonomy (45). It was nonphylogenetic, but it was the favored classification of microbes of the 1960s and 1970s.

Stanier was attuned to molecular biology. He maintained a close relationship with Jacques Monod and was at the Pasteur Institute around the time of the discovery of mRNA. Salvador Luria, Max Delbrück, and Joshua Lederberg were all in his intellectual circle of molecular biologists focusing on bacteria and their viruses. While none of those molecular biologists applied those methods to bacterial phylogeny, Stanier himself remained relatively aloof from molecular biology. As he commented in his autobiographical essay of 1980 (50): "The attentive reader will note that the dates of my activity [1940 to 1980] coincide, more or less precisely with the second great revolution in the history of biology: that of molecular biology. My own *activité ludique* wasn't in this heroic domain, perhaps as a result of a certain laziness, both physical and intellectual. However, I should like to emphasize that there really were microbiological treasures, simply waiting to be picked up. Let me recapitulate: the regulation of pigment synthesis by nonsulfur purple bacteria; the role of carotenoid pigments as agents of photoprotection; the life cycle of Caulobacter; the path of carbon in photoheterotrophy; the definition of bacteria as prokaryotes; the cyanobactacteria, like sleeping beauty, just emerging from a profound coma of 150 years. All this was virgin territory."

Stanier's interest in the possibility of recognizing natural

bacterial groups was also reawakened somewhat by new molecular methods. During the 1960s he worked to devise a system for the classification of the pseudomonads and the blue-green algae. In 1969 he explained his research agenda to Glazer, who was interested in spending a sabbatical year working with him (R. Y. Stanier to Alexander Glazer, 10 October 1969, National Archives of Canada, MG 31, accession J35, vol. 6): "One of the major problems which we have been exploiting over the past five years is the possibility of recognizing natural groups among the bacteria; and hence, of divising (sic) a system for the classification of such groups which would be expressive of their evolutionary relationships. Such work has been conducted largely, though not exclusively, on the aerobic pseudomonads, an important group of gram-negative bacteria. Even the formal phenotaxonomy of these organisms was chaotic when our work began, and we have had to do a great deal of purely descriptive taxonomic analysis in order to characterize the constituent species and species-clusters we then proceeded to ask what analyses at deeper levels—for example, the metabolic, regulatory and enzymological levels and the genetic level—would reveal about the relationships among the phenospecies."

Stanier further explained how he was also interested in trying to "put the study of the other major prokaryotic microbial group, the blue-green algae, on a scientific basis. Knowledge about these organisms had lagged, largely because so few of them have been isolated in pure cultures. We have been engaged for a number of years in isolating and purifying these organisms." Stanier's laboratory at Berkeley possessed the largest collection of pure cultures of blue-green algae in the world (about 50 strains), and he aimed to classify them in terms of nutrition, pigment composition, cellular fine structure, and DNA base composition. "So far," he wrote to Glazer in 1969, "we have not studied these organisms on a deeper level, but comparative studies on proteins would be very interesting. A good candidate is phycocyanin. . ."

In light of the new molecular approaches, in 1971 Stanier changed his views once more about the possibility of bacterial phylogeny (49): "In this essay, I shall develop the argument that we have at our disposal a variety of methods for ascertaining (within certain limits) *relationships* among the bacteria; and that where relationship can be firmly established, it affords a more satisfactory basis for the construction of taxa than does mere resemblance. As the philosopher G. C. Lichtenberg remarked 200 years ago, there is significant difference between *still* believing something and believing it *again*. It would be obtuse still to believe in the desirability of basing bacterial classification on evolutionary considerations. However, there may be solid grounds for believing it again, in the new intellectual and experimental climate which has been produced by the molecular biological revolution."

RELEASING THE PAST

New paradigms often emerge from outsiders, scientists who enter a field from a different discipline. The renaissance of microbial phylogenetics in the 1970s led by Carl Woese at the University of Illinois is exemplary. With the astute choice of the 16S rRNA as a phylogenetic probe and using the laborious molecular sequencing methods available in the 1970s, Woese

and his colleagues showed how one could achieve a comprehensive understanding of bacterial phylogeny and how to construct a universal tree experimentally (68). In doing so, they revealed distinct, separate lineages among bacteria, the archaebacteria, and the eubacteria in addition to a separate eukaryotic lineage (66). The rRNA method that Woese and his collaborators developed opened the whole field of microbiology to phylogenetic study. That technical knowledge was also used to investigate the origins of eukaryotes and the symbiotic origin of mitochondria and chloroplasts (22, 41, 43).

Woese was not immersed in the doctrines and dynamics of microbiology and the tumultuous discourse over the possibility of a natural classification of bacteria. It was the "deep" evolutionary questions that motivated him. Educated in biophysics and molecular biology, his interest lay in the genetic code and how it evolved. In order to understand the evolution of the translation process (indeed of any of the basic cellular machinery), he understood that one needed the framework of a universal phylogenetic tree. His great insight, the use of rRNA for phylogenetic purposes, was born of his pursuit of the evolution of the genetic code. Ribosomal RNA had all the right attributes. The cells of all organisms from bacteria to elephants need rRNAs to construct proteins. Therefore, their similarities and differences could be used to track every lineage of life. Ribosomes are also abundant in cells, so that their RNA was easy to extract. In short, the ribosome was of ancient origin, universally distributed, and functionally equivalent in all cells, making it the ideal organelle for following the course of evolution.

The work on 16S rRNA led to an upheaval in bacterial systematics and to major revisions of textbooks. Already by the end of the 1970s, Woese and his collaborators had sequenced the 16S rRNAs from about 60 kinds of bacteria and arranged them by genetic similarity (20, 64, 66). Their results tended to contradict the standard classification based on morphological similarities of bacteria. *Bergey's Manual* distinguished the gliding bacteria, the sheathed bacteria, the appendaged bacteria, the spiral and curved bacteria, and a host of families and such genera as *Flavobacterium* and *Pseudomonas*. But Woese and his collaborators concluded that these groups had no phylogenetic meaning; they were not genealogically coherent (monophyletic) groupings.

The reception of the new molecular approach to phylogeny and its findings was telling (and is a story in itself). Suffice it to say here that while the work was generally well received, this was not the case in some circles. Woese's approach to phylogenetics, the kind of data he assembled, and the conclusions drawn from them were met with great skepticism and apprehension by influential microbiologists and later (when a formal taxonomy was adduced therefrom) by some classical evolutionists, especially in the United States.

No aspect of the new phylogenetics attracted more attention than when Woese and George Fox, working in collaboration with Ralph Wolfe and William Balch, claimed to have discovered a "third form of life." Some microbiologists were simply incredulous about a third form of life from the outset when the National Science Foundation and National Aeronautics and Space Administration released a joint public statement on 3 November 1977. Wolfe received many phone calls on that morning. A front page article about a third form of life had appeared in *The New York Times*. Among those calls, the one

by Salvador Luria was "the most civil and free of four letter words." Wolfe recalled the episode (70):

> Luria: "Ralph, you must dissociate yourself from this nonsense, or you're going to ruin your career!" "But Lu, the data are solid and support the conclusions: they are in the current issue of PNAS [*Proceedings of the National Academy of Sciences*]." Luria: "Oh yes, my issue just arrived." "If you would like to discuss the paper after you have had a chance to look at it, give me a ring." He did not call again. I wanted to crawl under something and hide. Fortunately I was able to escape the hostility and left graduate students to cope, because my wife and I were leaving for Philadelphia to help celebrate her father's 90th birthday."

As Woese came to understand it, "the prokaryote-eukaryote dogma" had become firmly entrenched in the minds of biologists. And as a result, prokaryotes had falsely been assumed to be a monophyletic group. He and fox were forced to meet the murky semantics of the term prokaryote head-on when they introduced the term "archaebacteria" in 1977. "These 'bacteria,'" they wrote, "appear to be no more related to typical bacteria than they are to eukaryotic cytoplasms." (66) They commented that "Dividing the living world into *Prokaryotae* and *Eukaryotae* has served, if anything, to obscure the problem of what extant groupings represent the various primeval branches from the common line of descent. The reason is that eukaryote/prokaryote is not primarily a phylogenetic distinction, although it is generally treated so."

Woese and Fox argued there was no monolithic group of bacteria leading to eukaryotes; there was no prokaryote in that sense. Based on their comparisons of 18S rRNA of eukaryotic translation mechanisms, Woese and Fox argued that there was a separate line of descent that led to eukaryotes (apart from the symbiotic origin of mitochondria and chloroplasts). Thus, they put forward the concept of three "Urkingdoms" represented by three distinct lines of descent for the archaebacteria, eubacteria, and eukaryote. All three lineages would have diverged early from primitive cells in the throes of evolving their translation mechanisms (66, 67). These primitive hypothetical cells Woese and Fox called the progenotes (67).

Over the next 3 years, several other defining characteristics were grouped together to identify the three fundamental lineages, including the kinds of lipids, cell wall structure, the transcription mechanism, transfer RNAs, and the comparative 16S rRNA data, which for the first time allowed testing relationships among all "prokaryotes" (20). To emphasize that all prokaryotes do not share a common ancestry, in 1990, Woese, Otto Kandler, and Mark L. Wheeler renamed the archaebacteria the Archaea as a rhetorical term to counter the notion that they were "just more bacteria" (69).

To gain a better understanding of the attitudes of microbiologists and classical evolutionists and of the conceptual dogmas that he confronted, Woese reflected more and more on the history of microbial phylogeny (38, 65). He looked back on the 1950s and 1960s as "the Dark Age" of microbiology when phylogenetics was disavowed, led by Stanier and van Neil's declarations about it as a waste of time and when, in the "brave new molecular world evolutionary relationships counted for

naught" (65). Yet the prokaryote-eukaryote dichotomy had become unquestioningly accepted. Indeed, the rub for Woese was that Stanier and his colleagues illogically denied the possibility of large-scale phylogeny based on cell structure but still had no doubts about the monophyly of bacteria (the prokaryote) on the same grounds. Phylogeny by fiat had replaced experimentation and discussion, as he saw it.

The monophyly of the grouping had been assumed in all conceptions of the prokaryote. By the end of the 1960s, Woese suggested (65) that prokaryotes had been defined in some positive terms by using molecules and functions at the heart of the cell for which there were homologous among eukaryotes: chromosomal organization, regulatory mechanisms, and ribosome size. This might seem to make the old criticisms about its negative definition somewhat obsolete, thus confirming the prokaryote-eukaryote dichotomy as a true natural order of things. But there were problems. The molecular characterization of the prokaryote was based on a few "representative" bacteria, especially *Escherichia coli*, the favored organism of molecular biologists. This would not be an issue, of course, if one "knew" that the prokaryotes were monophyletic—that there existed only two basic types of cells on Earth. The confirmation of the prokaryote concept by molecular biology in the 1960s turned on a very tight circular argument. Belief in the prokaryote-eukaryote dichotomy fostered the notion that to understand bacteria, one only had to determine how *E. coli* differed from eukaryotes. Therefore, Woese argued that dichotomy only served to obscure profound differences among bacteria, and to hide from view microbiologists' nearly total ignorance of the relationships among bacteria (65): "Can you understand why I have such distaste for the prokaryote-eukaryote dichotomy? This is not the unifying principle that we all once believed it to be. Quite the opposite: it is a wall, not a bridge. Biology has been divided more than united, confused more than enlightened, by it. This prokaryote-eukaryote dogma has closed our minds, retarded microbiology's development, and hindered progress in general. Biological thinking, teaching, experimentation, and funding have all been structured in a false and counterproductive and dichotomous way."

Although Woese argued against the prokaryote concept, others continued to use it; they simply included the Eubacteria and the Archaea within the old dichotomy (2). The editors of the second edition of *The Prokaryotes* in 1992 offered historical comments similar to those of Woese when they introduced the new research and concepts in bacterial phylogeny based on 16S rRNA. They emphasized that although "Stanier and van Niel never actually defined the bacteria as a group," their seminal article of 1962 did emphasize the great diversity among bacteria. However, the editors argued, molecular biology played a counter role over subsequent decades in "narrowing of the scope of research on bacteria, since the incredible power and successes of molecular biology required intense study of only a few suitable model organisms. The hypothesis that there was a small group of typical bacteria whose mechanisms and processes were accurately representative of the bacteria as a whole became tacitly accepted" (2).

The first edition of *The Prokaryotes*, published in 1981, helped to broaden the focus on bacteria and "to recognize new mechanisms, new strategies for coping with the environment, newly expanded limits to the abilities of the microbe, and new

experimental systems" (2). In 1992 the editors reemphasized the diversity of the prokaryotes, "while adding the entirely new perspective of prokaryote phylogeny," which, they claimed, had hitherto been considered impossible. "The pioneering work of Carl Woese in cataloguing and sequencing the rRNA of prokaryotes has, for the first time in the history of biology, provided a means of establishing a truly phylogenetic system for living organisms—a goal previously thought impossible" (2).

No question that rRNA phylogenetics and subsequently genomics helped to radically shift interest from the few domesticates of genetics and molecular biology, such as *E. coli*, to the study of diverse forms. This dramatic difference is one of the chief characteristics of the era of genomics, which distinguishes itself from 20th century biology, a defining characteristic of which was progress through a few chosen model organisms. Those molecular biologists who used bacteria as a biotechnique were no more interested in the natural history and phylogeny of bacteria than *Drosophila* geneticists were interested in entomology. As two symbiosis researchers quipped recently, "It is a truth universally acknowledged that there are only two kinds of bacteria. One is E*scherichia coli* and the other is not" (19).

Still, debates over the history of bacterial taxonomy continued. Was bacterial phylogenetics previously thought impossible, as Woese and the editors of *The Prokaryotes* suggested? Often discussions centered over Stanier's deliberations and his effect on his contemporaries (21, 25). While Woese saw Stanier as a tragic figure who had given up on his hope for phylogenetics and who had discouraged its pursuit, others cast him as a hero who in fact fostered phylogenetics. In response to Woese's depiction, microbiologists John Ingraham and Horishi Nikaido commented (25): "Those of us who lived through the 1960s and 1970s as professional microbiologists know that this description does not reflect what actually happened. First Roger Stanier did not destroy the enthusiasm of microbiologists for phylogeny; he stimulated it. Perhaps his principal goal throughout his professional life was to make sense of the microbial world by organizing microorganisms into phylogenetically related groups and thereby to integrate microbiology into the rest of biology. Some molecular biologists may have thought that studying *E. coli* alone was enough, but Stanier and Douderoff were at the opposite end. They were always interested in and studied many diverse groups of microorganisms."

Ingraham and Nikaido suggest that Stanier had not really abandoned phylogeny but rather "sometimes let out cries of despair," frustrated by the inadequacy of the methods then available. As we have seen in this study, that feeling of hopelessness about microbial phylogeny was deep.

CONCLUDING REMARKS

The prokaryote-eukaryote dichotomy of the 1960s was not an astonishing formulation. It neither took scientists by surprise nor opened up radically new avenues of research. It was a rhetorical discovery, one which involved summoning lost words from a foreign scientist in an obscure publication urnal and synthesizing contemporary data based on molecular biology and electron microscopy. It was greeted with accolades because it seemed to resolve, once and for all, long-simmering

issues. It confirmed and clarified the differences between bacteria and blue-green algae on the one hand and viruses and the cells of protists, fungi, plants, and animals on the other. The belief in the monophyly of bacteria, moved by historical inertia and strengthened by molecular biology's model organism, resulted in a crowning achievement: the legitimation of the new kingdom Monera or of the superkingdom Prokaryota. The prokaryote-eukaryote dichotomy thus marks a signal moment in the development of biology.

Bacteriology's subject had remained undefined for a century when, in 1962, Stanier and van Niel addressed the concept of a bacterium. Yet they failed to provide a satisfactory concept of bacteria and their evolutionary relationships. In regard to constructing a natural classification of bacteria, they admitted that structural characteristics were no more useful than physiological properties. The prokaryote concept was, from its very inception, associated with pronouncements discouraging the possibility of bacterial phylogenetics, portraying it as a disreputable, unscientific pursuit. It is one of the great historical ironies therefore that the "prokaryote" was defined on the basis of microscopic structure.

I suggest that any resolution of this paradox must consider the changing place of the prokaryote in biological practice itself. The bacterium, which had lingered so long, floundering on the margins of biology proper, had moved to the center by mid-century. One "representative" prokaryote, *E. coli*, won distinction for the group in the husbandry of molecular biology, the heralded new queen of the life sciences. This fruitful marriage within biological research strengthened the call, for better or for worse, for a new kingdom. In effect, a fundamental change in the natural order of things reflected a change in biological order. And the hardening belief in a fundamental commonality among bacteria was legitimated when hastily naturalized within the realm Monera or superkingdom Prokaryotae.

Two concepts intermingled within the prokaryote neologism: one organizational and hierarchical, the other phylogenetic, presumed and untested, nested deep within the first. The prokaryote was characterized negatively in relation to structures that eukaryotes possessed, and even then only on the basis of one or few "prokaryotes" presumed to be "representative" of the group. A biological definition required coherent methods for comparing similarities and differences among all bacteria. Comparisons of 16S rRNA among thousands of different kinds of bacteria, leading to a universal evolutionary tree, showed the "prokaryotes" to be polyphyletic. Corroborated by cell wall structure, differences in the proteins involved in the translation processes and a host of other characteristic differences, the rRNA data indicated fundamental phylogenetic differences among bacteria, effectively refuting the concept of the "prokaryote" in both its organizational and phylogenetic senses.

Exposing these problems did not lead to a demise of the prokaryote concept. First, as noted in the five-kingdom proposal of the late 1960s, monophyly (though valued) was not considered an essential requirement for all of those who mapped phylogenetic realms. Second, some leading classical systematists did not shy from the negative characterizations used to identify the bacteria as the monolithic group prokaryotes. Indeed, Ernst Mayr remarked in 1998, when defending the unity of the "empire" Procaryotae, "The nonpossession

of a character is as positive a character in any traditional classification as is its possession (except in cases when the loss of a character can be determined with certainty)" (30). Those who demanded a (Darwinian) classification based on genealogy and who cautioned against defining large taxa in terms of negative characters had long warned of illusions resulting from convergent evolution. The reconstructed phylogenies based on 16S rRNA pointed up that illusion at the very moment that they moved bacteria to the center of evolutionary biology.

ACKNOWLEDGMENTS

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